CURRENTA GmbH & Co. OHG
Analytik
CHEMPARK, Gebäude K 46
51368 Leverkusen

1. Amendment to GLP-Final Report

Study Title: *Daphnia sp.*, Acute Immobilisation Test with PES Vorstufe 2342

Study number: 2010/0087/09

Test item: PES Vorstufe 2342

Date: 2011-05-25

CAS number: ---

Correction of the GLP-final report: ☒ **Addition to the GLP-final report:** ☐

Reason: The structural formula on page 10 is incorrect.

Correction / Addition: The structural formula is deleted without replacement.

Study director 2011-05-25 A. Neuhahn

Date / Signature

Enclosure: QS-statement for the amendment

CURRENTA GmbH & Co. OHG
Analytik
CHEMPARK, Building K46
D-51368 Leverkusen

Statement of the Quality Assurance on the Final Report

Key of the GLP-study: 2010/0087/09

Test substance: PES Vorstufe 2342

Title of the GLP-study: Daphnia sp., acute immobilisation test

This GLP-study was inspected by the quality assurance.
The dates of inspections and the dates of reports to the management and the study director are:

phase	date of inspection	date of report
final report review / 1. Amendment	30.05.2011	30.05.2011

The results shown in the final report on this study were inspected on the basis of the current SOPs/analytical methods. It is confirmed, that the report results to the best of our knowledge reflect the raw data of the study.

Quality assurance:

2011.05.30
Date

A. Seuß
Signature

Study Title

Daphnia sp., Acute Immobilisation Test with

PES Vorstufe 2342

Data Requirements / Test Guidelines:

EU method C.2 (2008)

OECD TG 202 (2004)

Author:

Astrid Neuhahn

Study completion date:

2011-05-23

Sponsor:

Bayer MaterialScience AG

BMS-IO-ST-PSRA-PRA

51368 Leverkusen

Germany

Testing facility:

CURRENTA GmbH & Co. OHG

Analytik

51368 Leverkusen

Germany

Monitor:

Dr. Ralf Werner

Bayer MaterialScience AG

BMS-IO-ST-PSRA-PRA

51368 Leverkusen

Germany

Laboratory Project Identification

Study No. 2010/0087/09

This page is intentionally left blank for the purpose of submitting administrative information that is required by regulations promulgated by various countries.

CONTENTS

1. GLP DECLARATION

This study was conducted in compliance with the OECD principles of Good Laboratory Practice (1999) and with the Principles of Good Laboratory Practice according to Annex I, German Chemical Law (2008).

Date / Signature

Study Director 2011-05-23 A. Neuhahn
(Neuhahn)

2. ARCHIVING

The original report, the study plan, and all raw data pertaining to this study are stored in the "GLP Archive, CURRENTA GmbH & Co. OHG, Analytik, CHEMPARK, Building Q 18, 51368 Leverkusen". A sample of the test item is stored in "GLP-Sample Store, CURRENTA GmbH & Co. OHG, Analytik, CHEMPARK, Building DA1, 41538 Dormagen".

This page is intentionally left blank for the purpose of submitting administrative information that is required by regulations promulgated by various countries.

3. QUALITY ASSURANCE STATEMENT

This report was audited by the Quality Assurance Unit CURRENTA Analytik, Quality Management at CURRENTA GmbH & Co. OHG and this statement confirms that the final report reflects the raw data.

The dates of Quality Assurance inspections and audits are given below.

Audits	Dates of QAU inspections	Dates of reports
study plan review	2011-04-08	2011-04-08
process based inspection	2011-01-11 2010/0142/02	2011-01-11
final report review (draft)	2011-05-20	2011-05-20
final report review	2011-05-25	2011-05-25

Date / Signature

2011-05-25 A. Senic

(Senic/ ~~Dr. Dörzbach-Lange/ Dr. Neupert~~)

4. STUDY TIME TABLE

Study initiation date:	2011-04-08
Study completion date:	2011-05-23
Start of experimental phase:	2011-04-11
End of experimental phase:	2011-05-11

5. GLP CERTIFICATE

Ministerium für Umwelt und Naturschutz, Landwirtschaft und Verbraucherschutz
des Landes Nordrhein-Westfalen

Postanschrift: 40190 Düsseldorf

Aktenzeichen: VI-3- 31.11.65.05

Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

Bayer Industry Services GmbH & Co OHG
Prüfeinrichtung BIS-SUA-Analytics
D-51368 Leverkusen

(unverwechselbare Bezeichnung und Adresse/Unequivocal name and address)

Prüfungen nach Kategorien (gemäß ChemVwV-GLP Nr. 5.3/OECD guidance)	Areas of Expertise (according ChemVwV-GLP Nr. 5.3/OECD guidance)
Kategorie 1	category 1
Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen	physical-chemical testing
Kategorie 4	category 4
Ökotoxikologische Prüfungen zur Bestimmung der Auswirkungen auf aquatische und terrestrische Organismen	environmental toxicity studies on aquatic and terrestrial organisms
Kategorie 5	category 5
Prüfungen zum Verhalten im Boden, im Wasser und in der Luft; Prüfungen zur Bioakkumulation und zur Metabolisierung	studies on behaviour in water, soil and air; bioaccumulation
Kategorie 8	category 8
Analytische Prüfungen an biologischen Materialien	analytical and clinical chemistry testing

Datum der Inspektion (Tag.Monat.Jahr)	Date of Inspection (day.month.year)
14. bis 16. September und 26. bis 28. Oktober 2005	on 14 until 16 September and on 26 until 28 October 2005
Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.	The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.
Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.	Based on the inspection report it can be confirmed, that this test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Düsseldorf, den 11. Januar 2006
Im Auftrag

(Prof. Dr. David)

Dienstsiegel/official-seal

Please note: Effective January 1st, 2008 the company name Bayer Industry Services GmbH & Co. OHG was changed to CURRENTA GmbH & Co. OHG

6. SUMMARY

A study was performed to assess the acute toxicity of PES Vorstufe 2342 to *Daphnia magna* STRAUS under static conditions.

The study was conducted in accordance with the Council Regulation (EC) No 440/2008, Method C.2 'Acute toxicity for *Daphnia*' (2008) which is equivalent to OECD Guideline for Testing of Chemicals No. 202 '*Daphnia sp.*, Acute Immobilisation Test' (adopted April 13, 2004).

The *Daphnia* were exposed to a limit test concentration of nominally 100 mg/L of PES Vorstufe 2342 dissolved in dilution water. Auxiliaries used to prepare the test media were an ultra turrax and a magnetic stirrer. Undissolved particles of the test item were removed using an aseptic filter.

Observations were made on the swimming ability and the immobilisation rate, respectively, after 24 and 48 hours of exposure. The following values were determined:

Time [h]	**EL 50 [mg/L]**
24	> 100
48	> 100

No toxic effects against *Daphnia* were observed at the limit of water solubility.

PES Vorstufe 2342 is insoluble or poorly soluble in water. Therefore a suitable selective and sensitive chromatographic method for the determination of the test item in aqueous solutions could not be established.

The results are expressed in terms of Effective Loadings (EL). As the test item is a multi constituent and no information about the correlation between molecular weight and the structural formula of the test item are available, a Water Accommodated Fraction (WAF) was used to test effects at a limit concentration of 100 mg/L, and no specific analysis was performed. With the sponsor's agreement, the content of the test item during the exposure period was verified by DOC determination.

The hardness of the dilution water used was 14.8 °dH (= 264.2 mg/L $CaCO_3$).

7. EXPERIMENTAL PROCEDURE

The method described in the Council Regulation (EC) No 440/2008, Method C.2 'Acute toxicity for *Daphnia*' (2008) which is equivalent to OECD Guideline for Testing of Chemicals No. 202 '*Daphnia sp.*, Acute Immobilisation Test' (adopted April 13, 2004) assesses the acute toxic effects (immobilisation) of various concentrations of a test item to a freshwater microcrustacean species.

The purpose of this method was to determine that concentration which causes a 50 % immobilisation rate (= EL 50) or, if conducted as a limit test, to determine the acute toxic effects at a maximum test concentration of 100 mg/L or at the limit of water solubility.

A range finding test (non-GLP) preceded the main test. It provided information about the range of concentrations which were used in the main test. The following nominal concentration of the test item was tested in the range finding test: 100 mg/L.

In the main test, *Daphnia* were exposed to the test item added to dilution water at a limit Effective Loading of nominally 100 mg/L for a period of 48 hours. At this concentration no toxic effects against *Daphnia* were observed at the end of the 48 hour study period, thus no statistical analysis was required to determine the EL50. Immobilisation rates were recorded at 24-hour intervals. Additionally any abnormal behaviour or appearance of the *Daphnia* was reported every 24 hours.

The main test was conducted as a static test with the test medium unchanged throughout the duration of the test.
During the test a temperature range of 18 - 22 °C was maintained in the test vessels, with a maximum temperature fluctuation of +/- 1 °C in each individual test. The temperature, the pH and the oxygen values were measured at the beginning and end of the test.

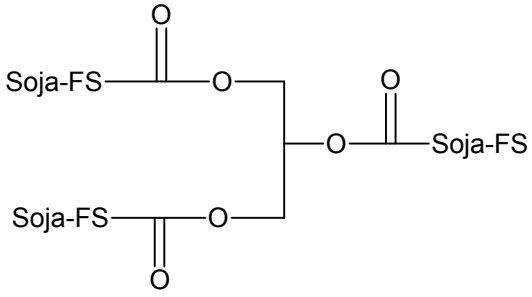
The following validity criteria of the test were met:

The immobilisation and other abnormalities in the controls did not exceed 10 % by the end of the test.
The dissolved oxygen concentration remained above 3 mg/L throughout the exposure period.

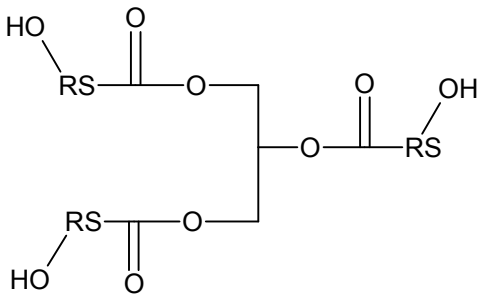
8. MATERIALS AND METHODS

8.1 Sample description

Test item	:	PES Vorstufe 2342
Chemical name	:	Castor Oil, reaction product with Soybean Oil
CAS name	:	--
CAS number	:	--
EC/NLP number	:	919-697-6
Sample provided by	:	Bayer MaterialScience AG
Empirical formula	:	--
Molecular mass	:	--
Structural formula	:	Reaction product of castor oil and soy bean oil (Transesterification)



Soja-FS = Soybean oil fatty acid



RS = Castor oil fatty acid

Batch number	:	LB06603520
Charge	:	--
Sample number	:	1199
Date of receipt	:	2010-04-27
Expiry date	:	2011-09-11
Purity	:	100 % (according to data of the sponsor)
Water solubility	:	0.0058 g/l
Density	:	0.95 g/cm3 at 20°C
Vapour pressure	:	ca. 4 hPa at 20°C
Stability of test concentration/s during exposure	:	Examined by chemical analysis (DOC) at 0 and 48 hours.

8.2 Test species

Name	:	*Daphnia magna* STRAUS, parthenogenetic females
Source	:	Strain of Bundesgesundheitsamt Berlin
Maintenance and Acclimatisation	:	A population of parthenogenetic females of synchronized age structure has been maintained for more than 15 years in the test facility under constant temperature conditions (20 +/- 1 °C) at a 16 : 8 hour light-dark photoperiod (light intensity: < 20 µE x m^{-2} x s^{-1}). The culture water (so-called 'M4 medium') was partly renewed once a week. The *Daphnia* were exclusively fed unicellular green algae (*Desmodesmus subspicatus*) 'ad libitum'. Mortalities of parent *Daphnia* during the culture period were recorded daily in a semi-quantitative way. The neonates were separated from their parent *Daphnia* by filtration prior to the acute test.

8.3 Culture and dilution water

Reconstituted water (so-called 'M4 medium' according to OECD 202) was used for the maintenance of the test animals and the preparation of stock and test solutions of the test item.

The total hardness of the dilution water, measured at test start, was 14.8 °dH (= 264.2 mg/L $CaCO_3$).

8.4 Apparatus

Analytical balance

pH meter

Oxygen meter

Incubator

Various glass material: volumetric flasks, beakers, watch glasses, pipettes etc.

8.5 Pre-treatment of test item and preparation of test item concentrations

A direct weighing was prepared to produce the only test concentration. 105.2 mg of the test item were added to 1 litre of dilution water, treated with an ultra turrax for 60 sec. at 8000 rpm and was then stirred for 24 h on a magnetic stirrer. Finally undissolved particles of the test item were removed by filtration using an aseptic filter with a pore size of 0.45 + 0.2 µm. The pH was measurd to be 7.9.

19 mL of the solution were taken and diluted with 1 mL of dilution water containing 10 daphnids resulting in the final concentration. For each test item concentration and the control 2 replicates were prepared.

8.6 Exposure conditions

Test vessels	:	50 mL glass beakers covered with watch glasses holding 10 neonates in 20 mL of test medium
Experimental design	:	1 test concentration plus 1 control
		10 neonates per vessel, 2 replicates per concentration/control
		no feeding during the exposure period
		static system
Method of initiation	:	neonates were placed in prepared media
Photoperiod	:	16 h light : 8 h dark
Temperature of incubation unit	:	20.4 to 20.5 °C

Aeration	:	none
Test item concentration/s	:	100 mg/L
Method of administration	:	direct weighing
Medium renewal	:	none
Duration of exposure	:	48 hours
Criteria of effects	:	The criterion of adverse effects used in this study was the item-induced alteration of the normal mobility behaviour and the loss of locomotory actions of the neonates, observed at 24 and 48 hours.

8.7 Chemical analysis

PES Vorstufe 2342 is insoluble or poorly soluble in water. Therefore a suitable selective and sensitive chromatographic method for the determination of the test item in aqueous solutions could not be established. With the sponsor's agreement, the content of the test item during the exposure period was verified by DOC determination.

Analytical Standards

Analytical Standard for Determination of Organic Carbon

Potassium hydrogen phthalate, dried at 105°C for 1 hour, purity > 99.9 %
Potassium hydrogen phthalate (nominal value: 2.125 g) was dissolved in water and made up to the mark in a 1000 mL volumetric flask to prepare a stock solution of 1000 mg Carbon per litre. Defined volumes of the stock solution were diluted with water to obtain standard solutions in the range of 5 to 300 mg/L.

Analytical Standard for Determination of Inorganic Carbon

Sodium carbonate, dried at 285°C for 1 hour, purity > 99.9 % Sodium hydrogen carbonate, dried for 2 hours over silica gel, purity > 99.9 %
Sodium carbonate (nominal value: 4.415 g) was dissolved in about 500 mL water. Sodium hydrogen carbonate (nominal value: 3.500 g) was added and made up to the mark in a 1000 mL volumetric flask to prepare a stock solution of 1000 mg Carbon per litre. Defined volumes of the stock solution were diluted with water to obtain standard solutions in the range of 15 to 150 mg/L.

Analytical Procedure

Principle

Total Carbon (TC) in water was oxidized to carbon dioxide by combustion. Inorganic Carbon (IC) was measured separately by acidification and purging. Total Organic Carbon (TOC) was calculated by the following equation:

$$TOC = TC - IC$$

As the bioavailable fraction of organic test items is more appropriately reflected by the Dissolved Organic Carbon (DOC), all biological test solutions were initially filtered through a membrane filter of a pore size of 0.45 µm before any further treatment was performed. In case of low DOC values (< 10 mg/L), DOC was measured after removing inorganic carbon by acidification and purging of carbon dioxide. In this case, DOC value was identical with TC.
Carbon dioxide was determined directly by infrared spectrometry.

Calibration

Linear calibration curves were established by analysing organic standard solutions and inorganic carbon solutions of at least three adequate concentrations. Typically, several calibration curves were used in order to cover the whole concentration range needed.

Limit of quantitation

2 mg/L DOC.

Analysis of samples

The biological test solutions were routinely measured on the day of sampling. If this was exceptionally not possible, the samples were stored in a refrigerator at 4 °C until the analysis was carried out. The biological test solutions were analysed in the same way as the calibration samples.

Evaluation of results

Injected samples were quantified by peak areas with reference to the respective calibration curve. The latter was obtained by correlation of peak area of the standard solutions to their corresponding concentration in mg/L. The correlation was performed using a linear function:

$$y = m \cdot x + b$$

y	= peak area of injected sample (counts)
x	= DOC of injected sample (mg carbon per litre)
m	= constant factor, slope of calibration curve
b	= intercept, point of intersection between calibration curve and y-axis

Factor 'Molecular weight / Organic C content': ----

Sampling schedule:

Control : at 48 hours

Test concentration : at 0 and 48 hours

8.8 Applied SOPs and methods

00159 V.2	Acute Daphnia Test
2011-0616101-07 D	Hardness of water
2011-0615201-07 D	DOC determination

Deviations : none

9. RESULTS

Table 1: Control

Abiotic parameters	**0 h**	**24 h**	**48 h**
Temperature [°C]	20.8	-----	21.1
Oxygen [mg/L]	8.4	-----	8.6
Oxygen [% saturation]	96	-----	95
pH value	7.9	-----	8.1
Immobilisation	**0 h**	**24 h**	**48 h**
Absolute	0	0	0
Cumulative	0	0	0
Cumulative [%]	0	0	0
Abnormalities *	**0 h**	**24 h**	**48 h**
Abnormal colouration	0/20	0/20	0/20
Abnormal swimming behaviour	0/20	0/20	0/20
Chemical analysis	**0 h**	**24 h**	**48 h**
DOC value [mg/L]	----	----	< 2

Comments: * *Daphnia* with effects / *Daphnia* mobile

Table 2: **100 mg/L**

Abiotic parameters	**0 h**	**24 h**	**48 h**
Temperature [°C]	20.7	-----	21.0
Oxygen [mg/L]	8.2	-----	8.4
Oxygen [% saturation]	95	-----	92
pH value	7.9	-----	8.0
Immobilisation	**0 h**	**24 h**	**48 h**
Absolute	0	0	0
Cumulative	0	0	0
Cumulative [%]	0	0	0
Abnormalities *	**0 h**	**24 h**	**48 h**
Abnormal colouration	0/20	0/20	0/20
Abnormal swimming behaviour	0/20	0/20	0/20
Chemical analysis	**0 h**	**24 h**	**48 h**
DOC value [mg/L]	< 2	----	< 2

Comments: * *Daphnia* with effects / *Daphnia* mobile

An analysis of the immobilisation rates gave the following results:

Time [h]	EL 0 [mg/L]	EL 100 [mg/L]	EL 50 [mg/L]
24	≥ 100	> 100	> 100
48	≥ 100	> 100	> 100

No toxic effects against *Daphnia* were observed at the limit of water solubility.

PES Vorstufe 2342 is insoluble or poorly soluble in water. Therefore a suitable selective and sensitive chromatographic method for the determination of the test item in aqueous solutions could not be established.

The results are expressed in terms of Effective Loadings (EL). As the test item is a multi constituent and no information about the correlation between molecular weight and the structural formula of the test item are available, a Water Accommodated Fraction (WAF) was used to test effects at a limit concentration of 100 mg/L, and no specific analysis was performed. With the sponsor's agreement, the content of the test item during the exposure period was verified by DOC determination.